IN VIVO METABOLISM OF Δ^1,17α-METHYLTESTOSTERONE IN MAN

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Our interest in the in vivo metabolism of Δ^1,17α-methyltestosterone (17α-methyl-17β-hydroxy-androst-1,4-diene-3-one) was stimulated by the successful isolation of urinary metabolites from 17α-methyltestosterone (17α-methyl-17β-hydroxy-androst-4-ene-3-one) and Δ^1-testololactone (17a-oxa-D-homoandrost-4-ene-3,17-dione). (1,2)

EXPERIMENTAL PROCEDURE

A 46 year old woman with advanced adenocarcinoma of the lung was given Δ^1,17α-methyltestosterone orally, one gram daily for 4 days. All the urine passed during these 4 days (3980 ml.) was refrigerated immediately after voiding and subsequently pooled. No preservative was added.

β-Glucuronidase Hydrolysis

From this pooled urine specimen 850 ml. aliquots were adjusted to pH 7.0. To each aliquot 170 ml. of chloroform were added and 170 ml. of 0.3 M phosphate buffer, pH 7.0, containing 33,333 units of bacterial β-glucuronidase. After the mixture was incubated for 24 hours at 37° C, 17,000 additional units of bacterial β-glucuronidase were added in 85 ml. of buffer and the mixture was incubated for an additional 48 hours.

Extraction Procedure

The urine was separated from the chloroform, readjusted to pH 6.0, placed in a Hershberg-Wolfe continuous extractor and extracted with chloroform for 24 hours. The chloroform extracts were combined and washed with 1/10 volumes (2 times) of 0.1 N NaOH and H_2O successively. The washed extracts were dried with anhydrous Na_2SO_4 and evaporated to dryness in vacuum.

Chromatography

Silica gel (Davison Chemical Corporation, 100-200 mesh) was prepared as previously described (3). The large (40 gram) silica gel chromatogram was performed in glass columns with an inside diameter of 3.0 cm. The small (2-5 gram) silica gel chromatograms were performed in glass columns with an inside diameter of 0.8 cm.

RESULTS

Purification of the Chloroform Residue

The dry residue was taken up in chloroform and chromatographed on 40 grams of silica gel with graded petroleum ether-chloroform, chloroform, and chloroform-ethanol elution.

The oily material obtained from the chloroform-ethanol (2 per cent ethanol) eluates was dissolved in methanol, combined and dried with a stream of nitrogen. The resulting residue was dissolved in a petroleum ether-chloroform (1:2) mixture and rechromatographed on 5 grams of silica gel, which was saturated with a petroleum ether-chloroform (1:2) mixture. Semicrystalline material was obtained in the chloroform eluates. The semicrystalline material was combined, dissolved in a petroleum ether-chloroform (1:2) mixture and rechromatographed on 2 grams of silica gel prepared in petroleum ether-chloroform (1:2). Crystalline material was obtained from the petroleum ether-chloroform (1:4)

eluates. The crystalline material was combined and recrystallized repeatedly from aqueous ethanol. Two hundred and thirty milligrams of crystalline material was obtained. The crystalline product had a melting point of 228-229° C. The compound was identified as Δ^1,6β-hydroxy-17α-methyltestosterone (17α-methyl-6β, 17β-dihydroxyandrost-1,4-diene-3-one) by its melting point, by the fact that the melting point of a mixture with authentic Δ^1,6β-hydroxy-17α-methyltestosterone was not depressed, and by its infrared spectrum.

Additional oily material (from the first chromatogram) eluted with petroleum ether-chloroform (1:4) was rechromatographed on 3 grams of silica gel prepared in a petroleum ether-chloroform (1:1) mixture. Semicrystalline material was obtained in the petroleum ether-chloroform (1:1) eluates. This semicrystalline material was combined, dissolved in a petroleum ether-chloroform mixture (1:1), and rechromatographed on 3 grams of silica gel as before, and crystalline material was obtained in the petroleum ether-chloroform (1:2) eluates. These eluates were combined and evaporated and the resulting crystalline material was recrystallized from aqueous ethanol. The resulting crystalline needles (60 mg.) melted at 222-224° C.

The ultraviolet absorption spectrum showed a maximum absorption peak at 243 millimicrons. The infrared spectrum had peaks of 6.0 mμ, 6.16 mμ and 6.24 mμ, thereby indicating the presence of the $\Delta^{1,4}$-diene-3-one grouping in ring A, and the -OH and -O-C regions of the spectrum were identical to that of Δ^1,17α-methyltestosterone. The infrared spectrum was slightly different from that of Δ^1,17α-methyltestosterone in the finger print region.

Microanalysis of the metabolite resulted in the following data:

Analysis calculated for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92

Analysis calculated for $C_{20}H_{28}O_2$: C, 79.95; H, 9.39

Found: C, 80.06: H, 9.45

Found: C, 79.14; H, 9.09

It can be seen that the empirical composition is much closer to that of Δ^1,17α-methyltestosterone ($C_{20}H_{28}O_2$) than to that of an oxygenated metabolite ($C_{20}H_{28}O_3$). It seems, therefore, that this metabolite (m.p. 222-224° C) is very similar (IR, UV, composition) in structure to Δ^1,17α-methyltestosterone (m.p. 164-166° C). At present, this metabolite is unidentified and work is in progress in an attempt to determine its structure.

DISCUSSION

In 1954 Burstein et al. (4) isolated 6β-hydroxy-cortisol (6β,11β, 17α, 21-tetrahydroxy-pregn-4-ene-3,20-dione) from the urine of a patient after administration of cortisol. They also demonstrated 6β-hydroxy-cortisol in urine of pregnant women (5). This work stimulated several investigators to look for the possibility of alternate pathways for the degradation of steroid hormones. A 6β-hydroxylating system has been shown to be present in the placenta by Berliner and Salhanick (6). Touchstone et al. (7) demonstrated that human adrenal slices are capable of the 6β-hydroxylation of cortisone (17,21-dihydroxy-pregn-4-ene-3,11,20-trione), and more recently Dominguez (8) showed that neoplastic testicular tissue is capable of 6β-hydroxylation of several steroids. Lipman et al. (9) showed that several tissues are capable of 6β-hydroxylation of cortisol (11β,17α,21-trihydroxy-pregn-4-ene-3,20-dione), and their findings suggest the liver as the major organ involved in this process. Lipman et al. also demonstrated that estrone (3-hydroxyestra-1,3,5,(10)-triene-17-one) enhances the 6β-hydroxylation of cortisol. Florini et al. (10) demonstrated that the major metabolic route of triamcinolone (9α-fluoro-11β,16α,17α,21-tetrahydroxy-pregna-1,4-diene-3,20-dione) in the dog is by 6β-hydroxylation.

In the present investigation we isolated two metabolites from Δ^1,17α-methyltestosterone. The major metabolite has been identified as Δ^1,6β-hydroxy-

17α-methyltestosterone. This work may indicate that one of the major pathways of metabolism for $\Delta^{1,4}$-3-keto,17-alkyl steroids in man is also through the 6β-hydroxylating system.

It also appears that Δ^{1},17α-methyltestosterone may proceed through a secondary route of metabolism, since we have an unidentified metabolite which has ring A intact. The possibility of Δ^{1},6α-hydroxy-methyltestosterone has been eliminated, since its infrared spectrum and melting point differ from that of the unknown. The carbon, hydrogen and oxygen analysis further tends to rule out the possibility of hydroxylation. At present we are inclined to think that it is an isomer of Δ^{1},17α-methyltestosterone. This problem is being investigated further.

SUMMARY

Δ^{1},6β-hydroxy-17α-methyltestosterone was isolated from the urine of a patient after administration of Δ^{1},17α-methyltestosterone. Another metabolite, in which ring A appears not to have been metabolized, was isolated but not as yet finally identified.

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